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ANNUAL RESEARCH PROGRESS REPORT

(FY 2007)

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHERN PLAINS AREA

GRAND FORKS, NORTH DAKOTA 58202

ANNUAL RESEARCH PROGRESS REPORT

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GRAND FORKS, NORTH DAKOTA 58202

NUTRITIONAL DETERMINANTS OF HEALTH

MANAGEMENT UNIT

5450-010-00

Project Number: 5450-51000-038-00D **Accession:** 0408766 **FY:** 2007
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: DAVID M KLURFELD **Principal Investigator:** WILLIAM T JOHNSON
Start Date: 07/21/2004 **Term Date:** 04/30/2009
National Programs: 107 N Human Nutrition
Title: DIETARY COPPER REQUIREMENTS FOR OPTIMAL CARDIOVASCULAR FUNCTION AND HEALTH
Period Covered From: 10/ 2006 **To:** 9/ 2007 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Overall, to determine, using animal models, whether copper (Cu) intakes consistent with those observed in humans can adequately support cardiovascular functions. To develop a strategy for assessment of marginal copper deficiency in animals; to use this strategy to determine biomarkers of copper status that are suitable for assessment of marginal status in humans. To determine the contribution of oxygen- and nitrogen-derived reactive species to the cardiomyopathy (metabolic, contractile) induced by Cu deficiency, and the dietary intakes at which this pathology occurs. To determine whether low Cu intakes consistent with those observed in humans can impair nitric oxide-dependent control of blood vessels and blood pressure regulation. To determine whether the oxidative stress induced by Cu deficiency affects homocysteine metabolism and, thereby, cardiovascular function, and whether such effects influence nitric oxide-dependent signal transduction and/or other mechanisms that affect atherosclerosis. To determine whether marginal Zn deficiency can exacerbate or unmask cardiovascular effects of sub-optimal Cu status by virtue of its role in oxidative/nitrosative metabolism.

1b. Approach (from AD-416)

Laboratory animals of varying ages and, in some cases, varying genetic makeup will be fed diets containing copper in severely deficient, marginally deficient or adequate amounts for varying periods of time. Based on comparisons to reliable invasive markers of copper status, non-invasive biomarkers will be tested for validity in assessing marginal copper deficiency. Tests of cardiovascular function and examinations of mechanism of depressed function will be made over ranges of copper status varying from severely-deficient to adequate. Cardiovascular functional measurements include heart contractile function, cardiac mitochondrial respiration and respiratory complex activity, blood vessel contractility and blood pressure. Atherosclerosis will be assessed by microscopic observation of blood vessels. Examination of mechanisms of depressed function will focus on the effects of oxidative stress and altered nitric oxide metabolism known to occur in copper deficiency. Such examination will include assessment of oxidative/nitrosative damage, altered nitric oxide signaling and altered homocysteine metabolism and will extend to measurement of transcription factors, mRNA and enzymes that influence and are influenced by oxidative stress and nitric oxide signaling.

2. Milestones for FY2007

1. Correlate organ copper content with semi-direct indicators of copper status; select best potential single biomarker(s).
   Milestone Not Met
   Reason not met: Critical vacancy (quantitative or qualitative deficiency in

personnel

2. Identify respiratory complexes affected by developmental copper deficiency.
Milestone Fully Met

3. Determine extent of oxidative modification of mitochondrial DNA by copper deficiency.
Milestone Not Met
Reason not met: Other (a reason for not meeting the Milestone other than the ones above)
Technical difficulties were encountered in developing the protocols for measuring oxidative modifications of DNA. These difficulties prevented completion of the milestone as scheduled. Development of the appropriate protocols is continuing.

4. Determine role of nitric oxide in impaired contractile function of copper deficiency.
Milestone Not Met
Reason not met: Critical vacancy (quantitative or qualitative deficiency in personnel

5. Determine relationship between nitric oxide, oxidative stress, homocysteine in atherosclerotic symptoms of copper deficiency.
Milestone Not Met
Reason not met: Critical vacancy (quantitative or qualitative deficiency in personnel

6. Clarify role of oxidative/nitrosative stress in zinc/copper interaction.
Milestone Fully Met

3. Progress Report

None.

4. Accomplishments

Copper supplementation reverses cardiac enlargement caused by pressure overload in mice.

In experimental rodents, copper deficiency causes cardiac enlargement that can be reversed by restoring normal copper status. However, little is known about the influence of dietary copper on cardiac enlargement caused by cardiovascular diseases that increase pressure overload. In mice, surgical constriction of the aorta produces chronic pressure overload and eventual cardiac enlargement and failure. It was found that supplementing the mice with about 3 times the normal dietary Cu requirement reversed cardiac enlargement and prevented heart failure in the mice subjected to chronic pressure overload. Copper supplementation improved cardiac enlargement in part by promoting the biogenesis of blood vessels in the heart. IMPACT: Cardiac enlargement and heart failure are a major cause of disability and death in humans. This study suggests that dietary copper requirements may increase in people with certain types of heart disease associated with cardiac enlargement and that supplemental copper may be beneficial for improving cardiac function in these people. [NP 107 Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance Measures 4.1.2: Define functions, bioavailability, interactions,

and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients in the food supply and provide that information in databases.]

Low copper intake during pregnancy but not during lactation results in the reduction of cytochrome c oxidase subunits encoded by both mitochondrial and nuclear DNA in cardiac mitochondria of the offspring.

One of our previous studies showed that reduced cardiac cytochrome c oxidase activity produced in the offspring of moderately copper-deficient rats resulted not from low copper intake during lactation, but from low intakes during pregnancy. The present study showed that the reduction in cytochrome oxidase activity resulting from low copper intake during pregnancy was accompanied by reductions in both the mitochondrial- and nuclear-encoded subunits of cytochrome c oxidase in cardiac mitochondria. Furthermore, the reduction in subunit content was not reversed by cross fostering the pups of copper-deficient dams to copper-adequate dams. This indicates that low copper intakes during pregnancy alters the programming of cardiac cytochrome c oxidase expression during fetal development. IMPACT: Dietary surveys indicate that pregnant women often do not meet the current recommended daily intake for dietary Cu. This finding indicates that moderately low Cu intake by pregnant women may alter the programmed development of the fetal heart in a manner that increases the risk of heart disease in their children because of impaired mitochondrial function. [NP 107 Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance Measures 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients in the food supply and provide that information in databases.]

5. **Significant Activities that Support Special Target Populations**

None.

6. **Technology transfer**

0 Number of new CRADAs and MTAs
0 Number of active CRADAs and MTAs
0 Number of invention disclosures submitted
0 Number of patent applications filed
0 Number of U.S. patents granted
0 Number of new commercial licenses granted
0 Number of web sites managed
5 Number of non-peer reviewed presentations and proceedings
1 Number of newspaper articles and other presentations for non-science audiences

Scientific Publications: **Log 115:**

1. Jiang, Y., Reynolds, C., Xiao, C., Feng, W., Zhou, Z., Rodriguez, W., Tyagi, S., Eaton, J.W., Saari, J.T., Kang, Y.J. 2007. Dietary copper supplementation reverses hypertrophic cardiomyopathy induced by chronic pressure overload in mice. Journal of Experimental Medicine. 204(3):657-666. 0000208706

2. Nielsen, F.H., Milne, D.B., Klevay, L.M., Gallagher, S., Johnson, L.K. 2007. Dietary magnesium deficiency induces heart rhythm changes, impairs glucose tolerance, and decreases serum cholesterol in post menopausal women. Journal of the American College of Nutrition. 26(2):121-132. 0000181721

3. Relling, D.P., Esberg, L.B., Johnson, W.T., Murphy, E.J., Carlson, E.C., Lukaski, H.C., Saari, J.T., Ren, J. 2007. Dietary interaction of high fat and marginal copper deficiency on cardiac contractile function. Obesity. 15(5):1242-1257. 0000199538

Project Number: 5450-51000-038-00D **Accession:** 0408766 **FY:** 2007

4. Saari, J.T., Reeves, P.G., Johnson, W.T., Johnson, L.K. 2006. Pinto beans are a source of highly bioavailable copper. Journal of Nutrition. 136:2999-3004. 0000199548

5. Saari, J.T., Wold, L.E., Duan, J., Ren, J., Carlson, H.L., Bode, A.M., Lentsch, A.B., Zeng, H., Schuschke, D.A. 2006. Cardiac nitric oxide synthases are elevated in dietary copper deficiency. Journal of Nutritional Biochemistry. doi:10.1016/j.jnutbio.2006.07.006. 0000195051

6. Uthus, E.O., Reeves, P.G., Saari, J.T. 2007. Copper deficiency decreases plasma homocysteine in rats. Journal of Nutrition. 137:1370-1374. 0000204889

7. Zeng, H., Saari, J.T., Johnson, W.T. 2007. Copper deficiency decreases complex IV but not complex I, II, III, or V in the mitochondrial respiratory chain in rat heart. Journal of Nutrition. 137:14-18. 0000199734

Approved: MCGUIRE MICHAEL R **Date:** 09/28/2007

Project Number: 5450-51000-039-00D **Accession:** 0409965 **FY:** 2007
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH **Principal Investigator:** CURTISS HUNT
Start Date: 08/01/2005 **Term Date:** 04/30/2009

National Programs: 107 N Human Nutrition

Title: MINERAL INTAKES FOR OPTIMAL BONE DEVELOPMENT AND HEALTH

Period Covered From: 10/ 2006 **To:** 9/ 2007 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Enhance the quality of life through establishing mineral intakes that support optimal bone health. Specifically, determine the amount of dietary calcium needed to maximize calcium retention and minimize bone resorption in postmenopausal women; determine the extent to which dietary protein, specific mineral elements (zinc, copper, magnesium, and boron) and prebiotics (inulin) interact with dietary calcium to affect bone metabolism.

1b. Approach (from AD-416)

Studies will utilize human subjects and animal models. Human studies will use the Mobile Nutrition Research Laboratory, the in-house Community Studies Unit, and the Metabolic Research Unit to conduct epidemiological supplementation, fortification, and controlled feeding experiments, respectively. In each case, subjects will be fed diets containing marginal to high amounts of mineral elements to determine how specific minerals, and interactions among them, affect bone structure (as determined by light microscopy, biomechanical assessment, and densiotometry) and biomarkers [urinary deoxypyridinoline, hemoglobin A1c, and C-reactive protein]). When relevant, the modifying influence of selected hormonal (e.g., estrogen deficiency) or diet compositional (e.g., inulin) factors will be examined.

2. Milestones for FY2007

1. Report on study on estimating the Ca requirement by titration; substitution of meta-analysis of existing human calcium balance data.
   Milestone Fully Met

2. Complete analyses from study on bone health during weight loss.
   Milestone Not Met
   Reason not met: Critical vacancy (quantitative or qualitative deficiency in personnel
3. Conduct the third of a three year study on bone health and copper and zinc supplementation.
   Milestone Fully Met

4. Conduct the third of a three year study of boron essentiality.
   Milestone Fully Met

Project Number: 5450-51000-039-00D Accession: 0409965 FY: 2007

5. Conduct post-growth phase of boron essentiality study.
   Milestone Substantially Met

6. Report on study on whether dietary boron improves calcium absorption.
   Milestone Substantially Met

7. Enroll subjects and conduct study on whether magnesium status affects calcium retention and bone resorption; substitution of meta-analysis of existing human magnesium balance data.
   Milestone Fully Met

8. Complete analyses from study on whether dietary inulin affects calcium absorption.
   Milestone Not Met
   Reason not met: Critical vacancy (quantitative or qualitative deficiency in personnel

3. Progress Report

None.

4. Accomplishments

New Estimation of the Calcium Requirement for Men and Women:
Calcium is an element that may be a risk factor for osteoporosis and current recommendations regarding the amount of calcium needed to support health and optimal biological function are based on sparse balance data. ARS scientists at Grand Forks, ND, established a new estimation of the average calcium requirement of healthy men and women by conducting secondary analyses of calcium balance data generated in 19 human controlled feeding studies at the Grand Forks Human Nutrition Research Center over the last three decades. The findings suggest a calcium requirement for healthy men and women (741 mg/d) that is lower than that estimated previously. This work will be very useful in setting the next Dietary Reference Intake for calcium. This accomplishment was conducted under National Program 107 Human Nutrition; goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining human requirements for known classes of nutrients.

Bone Loss Caused by Calcium Deficiency During Adolescence Can be Restored by Subsequent Adequate Calcium Intake:
Because women are at increased risk for bone fracture after menopause, it is recommended typically that teenage girls consume amounts of calcium during puberty sufficient to maximize their peak bone mass and thereby reduce the risk for later bone fracture. However, there is little information available whether poor calcium nutrition during adolescence has permanent effects on bone structure and strength. A cooperative study between scientists at the Grand Forks Human Nutrition Research Center, and the University of Oklahoma showed that the damaging effects of severe calcium deficiency on bone structure and strength during adolescence in female rats can be reversed completely by subsequent adequate calcium nutriture during early adulthood. This finding suggests that young women who consumed inadequate amounts of calcium during adolescence may be able to repair damage to their bones by increasing consumption of calcium-rich foods in early adulthood and thereby reduce their risk for bone fracture after menopause. This accomplishment was conducted under National Program 107 Human Nutrition; goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining human requirements for known classes of nutrients.

Project Number: 5450-51000-039-00D **Accession:** 0409965 **FY:** 2007

New Methodology Developed for Quantification of Normal Cell Death in Human White Blood Cells:
The ability of white blood cells to undergo controlled cell death is an essential feature in the maintenance and regulation of the immune response. ARS scientists at the Grand Forks Human Nutrition Research Center developed a method to assess the potential of white blood cells to undergo cell death in the intact whole blood sample. Whole blood collected from 30 free-living individuals was mixed with chemicals known to stimulate cell death. As cells begin to die, their DNA starts to break at characteristic points. Antibodies that target these broken ends were added and the blood sample was passed through an analyzer that counted each cell and the amount of attached antibody. This method appears capable of measuring the potential of white blood cells to go through a normal life cycle while minimizing unintentional physical damage to the cells during the assessment process. This accomplishment was conducted under National Program 107 Human Nutrition; goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining human requirements for known classes of nutrients.

5. **Significant Activities that Support Special Target Populations**

None.

6. **Technology transfer**

0 Number of new CRADAs and MTAs
0 Number of active CRADAs and MTAs
0 Number of invention disclosures submitted
0 Number of patent applications filed
0 Number of U.S. patents granted
0 Number of new commercial licenses granted
0 Number of web sites managed
7 Number of non-peer reviewed presentations and proceedings
1 Number of newspaper articles and other presentations for non-science audiences

Scientific Publications: **Log 115:**

1. Hunt, C. 2006. Boron in the typical diet: a physiological role for bone health, reproduction and insulin metabolism. Nutrition and the MD. 32(11):1-8. 0000199352

2. Hunt, C., Johnson, L.K. 2006. Magnesium requirements: new estimations for men and women by cross-sectional statistical analyses of metabolic magnesium balance data. American Journal of Clinical Nutrition. 84:843-52. 0000190781

3. Nielsen, F.H., Milne, D.B., Gallagher, S., Johnson, L.K., Hoverson, B. 2007. Moderate magnesium deprivation results in calcium retention and altered potassium and phosphorus excretion by postmenopausal women. Magnesium Research. 20(1):19-31. 0000189889

4. Roughead, Z.K. 2005. Influence of total diet on calcium homeostasis. Book Chapter. In: Weaver, C.M., Heaney, R.P., editors. Calcium in Human Health. New Jersey: Humana Press. p.191-208. 0000164959

Approved: MCGUIRE MICHAEL R **Date:** 09/28/2007

Project Number: 5450-51000-039-05N Accession: 0407993 FY: 2007
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Principal Investigator: FORREST H NIELSEN

Start Date: 03/01/2004 Term Date: 02/28/2009

National Programs: 107 N Human Nutrition

Title: HISTOMORPHOMETRIC AND BIOCHEMICAL ASSESSMENT OF THE POSSIBLE AUGMENTATION OF BONE HEALING AND REMODELING BY BORON

Period Covered From: 10/ 2006 To: 9/ 2007 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-4-0038F

Organization Name: UNIVERSITY OF SALTA

Progress and Outcomes:

1a. Objectives (from AD-416)

Enhance the quality of life through establishing mineral intakes that support optimal bone and joint health. Specifically, to confirm that boron is bioactive in osteogenesis and thus promotes bone growth and remodeling; and to establish dietary recommendations for boron that promote bone repair and maintains bone health.

1b. Approach (from AD-416)

Studies will use a mouse model of bone repair after injury. Mice will be fed boron-deficient and adequate diets. After about 5 weeks, surgical procedures will be performed for the purpose of evaluating peri-implant bone healing of the tibia and mandibular bone remodeling upon tooth extraction. Thirty days after the surgical procedures, the tibias and mandibles will be collected for histologic and histomorphometric examination. Tissues and plasma will be collected for the determination of indicators of bone formation and remodeling.

3. Progress Report

This report serves to document research conducted under a non-funded cooperative agreement between ARS and the National University of Salta, Argentina. Additional details of research can be found in the report for the parent project 5450-51000-039-00D Mineral Intakes for Optimal Bone Development and Health. The purpose of this research is to determine whether boron is bioactive in bone formation and thus promotes bone growth and remodeling. A research report has been prepared and will be submitted to Anatomical Record showing that boron deprivation compared to nutritional intakes of boron resulted in impaired alveolar bone healing after tooth extraction in rats because of reduced osteogenesis (bone formation). Another research report has been prepared and will be submitted to the Journal of Periodontal Research showing that boron deprivation impairs alveolar bone formation in rats. This research has also showed that boron deprivation decreased long bone stiffness in mice. The findings indicated that boron is required for optimal osteoblast activity (bone forming cells), and thus boron is needed for bone formation and strength, and low boron status impairs bone healing after injury. The ADODR monitored activities in this cooperative agreement through email and through personal contact at a scientific meeting.

Scientific Publications: Log 115:

Approved: MCGUIRE MICHAEL R Date: 08/20/2007

Project Number: 5450-51000-039-06S **Accession:** 0408592 **FY:** 2007
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH **Principal Investigator:** CURTISS HUNT
Start Date: 07/16/2004 **Term Date:** 09/30/2008

National Programs: 107 N Human Nutrition

Title: THE NUTRITIONAL ROLE OF BORON IN THE PREVENTION OF DIABETES

Period Covered From: 10/ 2006 **To:** 9/ 2007 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-4-0366

Organization Name: NORTH DAKOTA STATE UNIVERSITY

Progress and Outcomes:

1a. **Objectives (from AD-416)**

To contribute to basic nutrition research on the metabolic roles of boron, particularly as it may affect insulin binding/function.

1b. **Approach (from AD-416)**

Nutritional requirements and dietary recommendations for the trace mineral boron will be investigated in cell culture and animal models and humans, through application of dietary boron deprivation, supplementation, and repletion. The human studies will be conducted with appropriate review and approval by the respective Institutional Review Boards used by the University and ARS.

3. **Progress Report**

This report serves to document research conducted under specific cooperative agreement between ARS and North Dakota State University. Details of this project can be found in the report for the parent CRIS 5450-510000-039-00D.

To better define the mechanism by which dietary boron decreased plasma insulin levels and increased insulin sensitivity, we have successfully conducted an analysis of muscle insulin receptor concentrations in a study to extend our previous findings. The findings were summarized in the report for the in-house parent project. ADODR monitoring activities to evaluate research progress included electronic mail correspondance.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 08/20/2007

Project Number: 5450-51000-039-07T **Accession:** 0408848 **FY:** 2007
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Principal Investigator:** FORREST H NIELSEN

Start Date: 07/01/2004 **Term Date:** 04/30/2008

National Programs: 107 N Human Nutrition

Title: EFFECT OF ARGININE SILICATE INOSITOL COMPLEX ON BONE AND JOINT HEALTH

Period Covered **From:** 10/ 2006 **To:** 9/ 2007 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 04-5450-4-0415

Organization Name: NUTRITION 21, INC.

Progress and Outcomes:

1a. Objectives (from AD-416)

To determine whether silicon as an arginine silicate inositol complex is useful in overcoming the lack of dietary silicon that may lead to pathological changes in bone or connective tissue including bone cartilage (e.g., osteoarthritis) and thus result in bone erosion or loss.

1b. Approach (from AD-416)

Dark agouti rats that are especially susceptible to chronic and severe forms of autoimmune arthritis will be injected with type II collagen to induce arthritis after being fed silicon-deficient and silicon supplemented (as arginine silicate inositol complex) for 5 weeks. The development of arthritis will be evaluated by a macroscopic scoring system and joint swelling measurements. During arthritis development urinary bone breakdown and plasma inflammatory variables will be determined. Five weeks after injection of collagen II the animals will be killed for the determination of blood and bone variables associated with arthritis development and bone loss. Bones will be examined histologically to evaluate the effect of dietary silicon on cartilage erosion and bone resorption.

3. Progress Report

This report serves to document research conducted under a Trust Fund Cooperative Agreement between ARS and Nutrition 21, Inc., Purchase, NY. Additional details of research can be found in the report for the parent project 5450-51000-039-00D Mineral Intakes for Optimal Bone Development and Health. The purpose of this research is to determine whether silicon as a novel arginine silicate inositol complex or sodium metasilicate prevents any silicon deprivation-enhanced undesirable changes in markers of bone and connective tissue metabolism induced by pro-inflammatory agents. A report was submitted to Journal of Trace Elements in Medicine and Biology suggesting that, in rodents, physiological amounts of silicon promote the immune response; sex may influence the response to dietary silicon; and both organic silicon complexes (i.e., arginine silicate inositol) and inorganic silicon are similarly effective in preventing changes in inflammation induced by silicon deprivation.

Progress in this project was communicated to Nutrition 21, Inc. by the ADODR through telephone calls, emails, and personal contact at scientific meetings.

Scientific Publications: **Log 115:**

Project Number: 5450-51000-039-07T Accession: 0408848 FY: 2007

Approved: MCGUIRE MICHAEL R Date: 08/20/2007

Project Number: 5450-51530-009-00D **Accession:** 0408299 **FY:** 2007
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Principal Investigator:** HENRY C LUKASKI

Start Date: 04/03/2004 **Term Date:** 04/02/2009

National Programs: 107 N Human Nutrition

Title: MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

Period Covered From: 10/ 2006 **To:** 9/ 2007 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Improve health and enhance quality of life by determining, for healthy and at-risk populations (e.g., school-aged children, rural elderly, Native Americans), mineral intakes that promote optimal physiological and psychological development, function and health; develop new functional bases for establishing mineral element requirements; identify mechanisms of action; and determine the influence of sex, age, genetic, lifestyle and environmental factors on mineral element requirements. Develop and implement health promoting interventions for prevention of obesity and co-morbidities in American Indian population in the upper Midwest.

1b. Approach (from AD-416)

Dietary intakes and biochemical indices of mineral status will be related to physiologic (e.g., body composition, weight maintenance, physical fitness, energy metabolism, brain and cardiac function) and psychological (e.g., cognition, emotional and social adjustment, school/work performance) measures to determine roles of specific minerals in supporting optimal function and development. A Mobile Nutrition Research Laboratory, Community Studies Unit, and a residential Metabolic Research Unit will be used to conduct epidemiologic, supplementation, fortification, and controlled feeding studies, respectively with healthy and at-risk subjects (e.g., school-aged children, rural elderly, Native Americans). Use qualitative assessment methods (focus groups and in-depth interviews) and surveys to develop and implement social ecological, culturally-sensitive and scientifically sound interventions in American Indian communities. Randomized controlled trials will evaluate the effects of graded intakes of minerals, such as iron, zinc, copper, mangesium and boron, and mediating factors (e.g., genotype, controlled stressors). Animal studies will be used to determine the mechanisms of action of functional outcomes. Studies will involve university, industry and government collaboration.

2. Milestones for FY2007

1. Conduct study of zinc supplementation of adolescents at second site. Anticipated outcome: determine the effects of zinc supplementation at recommended and higher amounts on growth, development, body composition, cognition, social adaptation, and physical fitness of youth.
   Milestone Not Met
   Reason not met: Critical vacancy (quantitative or qualitative deficiency in personnel

Project Number: 5450-51530-009-00D Accession: 0408299 FY: 2007

2. Conduct study of magnesium requirements of postmenopausal women with outcomes of magnesium nutritional markers, inflammatory responses and cardiovascular risk factors.
Milestone Substantially Met

3. Plan and initiate study of copper on adaptation to increased physical activity of out-bred rats with different phenotypes for aerobic capacity.
Milestone Substantially Met

4. Develop nutrient data base of commodity and traditional Native foods.
Milestone Substantially Met

5. Initiate community focus groups to identify and report perceived health concerns/needs of tribal communities.
Milestone Fully Met

6. Analyze samples and data from observational study of the elderly; report results.
Milestone Substantially Met

7. Plan experimental protocol for study of effects of graded zinc intake on adaptation to increased physical activity in humans.
Milestone Not Met
Reason not met: Critical vacancy (quantitative or qualitative deficiency in personnel

3. **Progress Report**

None.

4. **Accomplishments**

Health Claims on Labels and Nutrient Composition of Common Foods in Grocery Stores.

The labels of food products provide information describing the nutrient contents and health claims in some instances. The public may purchase such a product with the understanding that it is healthful regardless of the composition of the remaining contents. However, the use of such statements may not reflect other contents of these products that may be not health-promoting. In a survey of local grocery stores, more than 57,000 products were examined and half had nutritional marketing information. Almost half of these products had excessive amounts of saturated fat (+/-20% daily value), sodium (+/-20% daily value) and/or added sugar (+/-6 g per serving for non-fruit/milk-based products and +/-21 g per serving for fruit/milk-based products). Thus, nearly one in four products had both marketing and less than healthful contents. Similarly, more than 9000 products were targeted to children (e.g., child-oriented graphics, cartoon or celebrity characters, toys/prizes, etc), and 78% had a nutritional marketing label. More than 60% of the products marketed to children were high in saturated fat, sodium and/or sugar. Nearly half of the products targeted to children had nutritional marketing labels and less than healthful contents. IMPACT: Advertisement of food products can provide misleading information that the public can interpret as health-promoting. Educational programs that instruct children and adults to evaluate all information on labels can be useful in reducing excess consumption of energy and thus promote healthy body weight. NP 107 National Action Plan Component 6: Prevention of Obesity and Disease: Relationships between Diet, Genetics, and Lifestyle.

Project Number: 5450-51530-009-00D **Accession:** 0408299 **FY:** 2007

Boron Deprivation Changes in Rat Behavior Attenuated by Omega-3 Fatty Acids.

Controversy exists regarding the effects of dietary polyunsaturated fatty acids (PUFA) on behavior. Some studies with rats show that, compared to diets high in n-6 PUFA, diets high in long chain n-3 PUFA, improve rat behavior, but other studies do not. The inconsistency in findings may be the result of different intakes of another food component, such as boron, shown to affect similar functions as long chain n-3 PUFA. ARS scientists at Grand Forks, ND, found that boron deprivation of rats fed a diet high in n-6 PUFA changed rat voluntary movement and exploratory behavior such that they were less active. A diet high in n-3 PUFA reduced the effects of boron deprivation. IMPACT: Diets high in both boron and n-3 PUFA, rather than either alone, improve behavior and activity when dietary boron is restricted and n-6 PUFA intake is high. This finding provides evidence of the need to consume fruits and vegetables consistent with national guidelines for health promotion. [NP 107 National Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance Measure 4.1.2: Define functions, bioavailability, interactions and human requirements for known, emerging, and new classes of nutrients in the food supply and provide that information in databases.

5. **Significant Activities that Support Special Target Populations**

Scientists in the unit continue to work with American Indians to develop successful partnerships and to promote research on health promotion. Examples include initiation of a Cultural Awareness Workshop at United Tribes Community College, attended by the researchers, technicians and administrators from throughout the Northern Plains Area. These activities directly support Grand Forks Human Nutrition Research Center programs to improve the nutrition and health of this at-risk and underserved population in our region, and facilitate accomplishment of the milestone related to health promotion and obesity prevention in American Indian communities.

In addition, an important Specific Cooperative Agreement was completed to promote collaborative research partnerships with Cankdeska Cikana Community College (Spirit Lake Reservation). This agreement seeks to formalize relationships to initiate discussion geared to develop culturally-appropriate activities and intervention to promote health and prevent obesity and diabetes among American Indians in the Northern Great Plains. One project completed during this past year used qualitative methods to identify and characterize barriers and facilitators associated with potential nutrition and lifestyle changes to achieve and maintain a healthy body weight and reduce risk of chronic diseases and mitigate disease symptoms at Cankdeska Cikana Community College (Spirit Lake Reservation). This work was accomplished in the subordinate project (#5450-51530-009-05S), and is related to NP 107, Nutrient Requirements; Component 7: Health Promoting Intervention Strategies for Targeted Populations.

6. **Technology transfer**

0	Number of new CRADAs and MTAs
0	Number of active CRADAs and MTAs
0	Number of invention disclosures submitted
0	Number of patent applications filed
0	Number of U.S. patents granted
0	Number of new commercial licenses granted
0	Number of web sites managed
12	Number of non-peer reviewed presentations and proceedings
2	Number of newspaper articles and other presentations for non-science audiences

Scientific Publications: **Log 115:**

1. Colby, S.E. 2007. Peer-led theater as a nutrition education strategy. Journal 0000190176

Project Number: 5450-51530-009-00D **Accession:** 0408299 **FY:** 2007

of Nutrition Education and Behavior. 39:48-49.

2. Lukaski, H.C. 2007. Effects of chromium (III) as a nutritional supplement. In: Vincent, J.B., editor. The Nutritional Biochemistry of Chromium (III). Amsterdam, The Netherlands: Elsevier. B.V. p. 71-84. 0000181628

3. Lukaski, H.C. 2007. Recommended proportions of carbohydrates to fats to proteins in diets. In: Driskell, J.A., editor. Sports Nutrition: Fats and Proteins. Florida, Boca Raton: CRC Press. Lord and Francis Group. p. 357-372. 0000201276

4. Lukaski, H.C., Hall, C.B., Siders, W.A. 2007. Assessment of change in hydration in women during pregnancy and postpartum with bioelectrical impedance vectors. Nutrition. 23:543-550. 0000206166

5. Lukaski, H.C., Siders, W.A., Penland, J.G. 2007. Chromium picolinate supplementation in women: effects on body weight, composition, and iron status. Nutrition 23:187-195. 0000190704

6. Nielsen, F.H. 2006. Boron, manganese, molybdenum, and other trace elements. In: Bowman, B.A., Russell, R.M. editors. Present Knowledge and Nutrition, Ninth Edition. Washington, DC: International Life Sciences Institute Press. Vol I, p. 506-526. 0000183471

7. Nielsen, F.H. 2007. Summary: the clinical and nutritional importance of chromium - still debated after 50 years of research. In: Vincent, J.B., editor. The Nutritional Biochemistry of Chromium (III). Amsterdam, The Netherlands:Elsevier B.V. p. 265-276. 0000191696

8. Nielsen, F.H., Lukaski, H.C. 2006. Update on the relationship between magnesium and exercise. Magnesium Research. 19(3): 180-189. 0000193550

Approved: MCGUIRE MICHAEL R **Date:** 09/28/2007

Project Number: 5450-51530-009-03N Accession: 0409328 FY: 2007
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH Principal Investigator: HENRY C LUKASKI
Start Date: 05/26/2005 Term Date: 03/31/2009
National Programs: 107 N Human Nutrition
Title: ASSESSMENT OF MINERAL LOSSES IN SWEAT DURING PHYSICAL ACTIVITY
Period Covered From: 10/ 2006 To: 9/ 2007 Final Report? No
Terminate in Two Months? No
Agreement Number: 58-5450-5-0107N
Organization Name: GATORADE SPORTS SCIENCE INSTIT.

Progress and Outcomes:

1a. Objectives (from AD-416)

This project will evaluate the feasibility of determining sweat losses of mineral elements in humans during periods of controlled physical activity. This study is an initial effort to develop a valid and reliable method to determine surface mineral losses. This method is needed to improve assessment of mineral nutritional needs of physically active people.

1b. Approach (from AD-416)

The cooperator will recruit and enroll skilled athletes to participate in the approved study to determine the effects of physical activity on body sweat losses. The cooperator will provide unique sweat collection apparatus and position them on various sites of the body before controlled physical activities, monitor mineral-containing beverage intake, then remove the sweat collection devices. Sweat will be extracted and sent to the USDA, ARS Grand Forks Human Nutrition Research Center for determination of mineral concentrations. Data will be shared between ARS and cooperator scientists and prepared for publication in a scientific journal.

3. Progress Report

This report serves to document research conducted under non-funded cooperative agreement between ARS and Gatorade Sport Science Institute. Additional information can be found in the report for the parent project 5450-51530-009-00D Micronutrient Roles in Physiology and Health.

Progress includes the development of an appropriate reference material, pooled sweat reference, for use in quality control assessment of the analytical method to determine mineral concentrations in human sweat.

The mineral composition of sweat collected during physical activity among youth is unknown. Sweat was collected during practice from male and female elite youth soccer players in different age groups (9-10 y, 11-12 y, and 14-15 y) to ascertain if mineral concentrations were different by gender and compared to data from adults. Sodium concentrations tended to increase with age, and males had greater concentrations of sodium in sweat than females. Magnesium concentrations in sweat of these youth were less than the lowest concentrations reported in adults also exercising.

Activities were monitored by using monthly conference calls. Collaborators jointly

Project Number: 5450-51530-009-03N **Accession:** 0409328 **FY:** 2007

organized a symposium at the American College of Sports Medicine and presented findings of this research.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 08/20/2007

Project Number: 5450-51530-009-04N Accession: 0410137 FY: 2007
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH Principal Investigator: GERALD F COMBS
Start Date: 03/09/2006 Term Date: 09/30/2010

National Programs: 107 N Human Nutrition

Title: MINERAL NUTRITION RESEARCH

Period Covered From: 10/ 2006 To: 9/ 2007 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-6-0101N

Organization Name: US ARMY RES INST ENVIR MEDICINE

Progress and Outcomes:

1a. Objectives (from AD-416)

Collaborator in planning, implementation and reporting of research on the effects of minerals on human nutritional needs and physical and psychological performance.

1b. Approach (from AD-416)

Human volunteers will be studied under a variety of dietary conditions and biochemical and functional parameters will be measured.

3. Progress Report

This report serves to document research conducted under a non-funded cooperative agreement between ARS and the United States Army Research Institute for Environmental Medicine (USARIEM). Additional details of research can be found in the report for the parent project, 5450-51530-009-00D, Micronutrient Roles in Physiology and Health.

The project represents the commitment of the Grand Forks Human Nutrition Research Center and the United States Army Research Institute for Environmental Medicine (USARIEM) to collaborate in studies of mutual value in the general area of mineral nutrition. The focus of efforts this year was in the development of effective methods that minimize contamination for collecting and analyzing human sweat losses. Methodological development was initiated by the Grand Forks Human Nutrition Research Center to analyze the micronutrient contents of human sweat samples, with particular attention to zinc, copper, iron and calcium, magnesium, sodium, potassium and chloride. Collaborative efforts were made to develop effective means of collecting human sweat, particularly from physically active individuals under varying, controlled environmental conditions at USARIEM. Plans were made for a planning meeting of scientists from each of the cooperators' locations to be held in early FY08.

Uncertainty exists regarding the effect of sustained sweating on sweat mineral composition. A series of studies determined the effect of multiple hours of exercise in the heat on mineral concentrations of sweat collected on the back of soldiers adapted to a constant hot environment under different conditions (hot and dry compared to hot and humid). Responses were similar regardless of heat exposure conditions in the heat-acclimatized soldiers. Compared to the initial exposure, sweat concentrations of sodium, potassium, calcium, magnesium, and copper did not change with subsequent exposures. However, sweat zinc concentrations decreased

Project Number: 5450-51530-009-04N **Accession:** 0410137 **FY:** 2007

significantly.

Investigators participated in weekly conference calls, discussed experimental plans, reviewed data, and met at each research location to discuss findings, which were presented at a national conference. The published abstract is listed in the report for the parent project (5450-51530-009-00D).

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 08/20/2007

Project Number: 5450-51530-009-05S Accession: 0411236 FY: 2007
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH Principal Investigator: GERALD F COMBS
Start Date: 09/19/2006 Term Date: 08/31/2011
National Programs: 107 N Human Nutrition
Title: HEALTH PROMOTION IN AMERICAN INDIAN COMMUNITIES
Period Covered From: 10/ 2006 To: 9/ 2007 Final Report? No
Terminate in Two Months? No
Agreement Number: 58-5450-6-0351
Organization Name: CANKDESKA CIKANA COMM COLLEGE

Progress and Outcomes:

1a. Objectives (from AD-416)

The broad objective of this cooperative research is to develop information useful in promoting health through improved nutrition and lifestyles. The specific objectives are to:
1. Develop understanding of the relationships of diet, lifestyle, and the prevalence of chronic diseases, particularly obesity, diabetes, and cardiovascular disease in American Indian peoples;
2. Identify health needs, and the barriers to and facilitators of meeting those needs in American Indian communities;
3. Determine the efficacy of community-based, health-promoting, intervention strategies in American Indian communities; and,
4. Increase research cooperation between American Indian colleges and USDA-ARS.

1b. Approach (from AD-416)

Identification and characterization of barriers to and facilitators of eating healthy diets and engaging in healthy lifestyles will be accomplished through a series of focus groups in American Indian communities. Focus groups will be designed to also identify and prioritize community needs as potential mediating factors. Subsequently, health-promoting intervention strategies will be developed and evaluated based on identified barriers and facilitators in the context of community needs and priorities. A research involving human subjects will be conducted with appropriate review and approval by the respective institutional review boards used by Cankdeska Cikana Community College and ARS.

3. Progress Report

This report serves to document research conducted under a Specific Cooperative Agreement between ARS and the Cankdeska Cikana Community College. Additional details of research can be found in the report for the parent project 5450-51530-009-00D, Micronutrient Roles in Physiology and Health.

This project represents the commitment of the Grand Forks Human Nutrition Research Center and the Cankdeska Cikana Community College to collaborate in studies of mutual value to promote the health of American Indians. Focus groups of American Indians were assembled to develop formative information on facilitators and barriers to compliance with a national guideline for diet and physical activity, Dietary Guidelines for Americans.

Project Number: 5450-51530-009-05S **Accession:** 0411236 **FY:** 2007

Activities were monitored by frequent site visits at Cankdeska Cikana Community College and regular conference calls including discussions for planning activities among the investigators.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 08/20/2007

Project Number: 5450-51530-009-07N **Accession:** 0411613 **FY:** 2007
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH **Principal Investigator:** HENRY C LUKASKI

Start Date: 02/01/2007 **Term Date:** 04/02/2009

National Programs: 107 N Human Nutrition

Title: DIET AND EXERCISE ON PROTEIN EXPRESSION IN MUSCLE

Period Covered **From:** 10/ 2006 **To:** 9/ 2007 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-7-0110N

Organization Name: US ARMY RES INST ENVIR MEDICINE

Progress and Outcomes:

1a. Objectives (from AD-416)

To identify micro-nutrient responsive proteins in muscle and other tissues obtained from rodent models.

1b. Approach (from AD-416)

Laboratory rodents of varying ages and, in some cases varying genotypes and phenotypes, will be fed diets containing micronutrients in marginally-deficient and adequate amounts and either exposed to physical training or untrained. Comparisons will be made among established biochemical and physical markers of nutritional status and expression of proteins in selected tissues to determine impacts of sub-clinical micronutrient deficiencies. Emphasis will be muscle mitochondrial complexes and signal transduction in mitogenesis and angiogenesis.

3. Progress Report

This report serves to document research conducted under a non-funded cooperative agreement between ARS and the United States Army Research Institute for Environmental Medicine (USARIEM). Additional details of research can be found in the report for the parent project 5450-51530-009-00D, Micronutrient Roles in Physiology and Health.

The project represents the commitment of the Grand Forks Human Nutrition Research Center and the United States Army Research Institute for Environmental Medicine (USARIEM) to collaborate in studies of mutual value in the broad area of interaction of diet and physical activity on expression of proteins that regulate structural and functional adaptations of skeletal muscle and other key organs. Development of procedures and implementation of use of novel technology (E-PAGE system) was initiated by the Grand Forks Human Nutrition Research Center to analyze tissue samples to determine proteins fundamental in stimulation of mitochondria to increase in number and new blood vessels to grow in muscle. Collaborative efforts were made to develop standardized experimental protocols, preparative methods and analytical procedures. Regular teleconferences were held to discuss project plans, review progress, and discuss data. Meetings were conducted at the collaborator's laboratory and at national conferences to review information, plan additional experiments and review preliminary findings. Plans were made for additional meetings of scientists from each of the cooperators' locations to be held in early FY08.

Scientific Publications: **Log 115:**

Project Number: 5450-51530-009-07N Accession: 0411613 FY: 2007

Approved: MCGUIRE MICHAEL R Date: 08/20/2007

MICRONUTRIENT ABSORPTION AND METABOLISM

MANAGEMENT UNIT

5450-020-00

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2007
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH **Principal Investigator:** JANET ROSS HUNT
Start Date: 01/15/2004 **Term Date:** 01/14/2009

National Programs: 107 N Human Nutrition

Title: MINERAL UTILIZATION AND BIOAVAILABILITY IN THE 21ST CENTURY, WITH CHANGING DIETS AND AGRICULTURAL PRACTICES

Period Covered **From:** 10/ 2006 **To:** 9/ 2007 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

The general objective is to determine how current and proposed changes to the American diet that may adversely affect intake and/or how bioavailability of the essential mineral nutrients can be modified to enhance trace element nutrition, with emphasis on selenium (Se), iron (Fe), zinc (Zn), and copper (Cu). Specific objectives are:

Objective 1: Determine how shifts in agricultural and dietary practices, such as the availability of functional/genetically modified foods and emphasis on plant-based diets with reductions in meat consumption will impact the intake, bioavailability, and dietary requirements of minerals. This objective will address the production of foods with enhanced bioactive Se compounds, and assess their ability to enhance health, especially by controlling oxidative stress and reducing cancer risk. The impact of organic farming methods will also be assessed (Finley). It will also address the practical impact of dietary changes that emphasize plant-based diets on meeting nutritional needs for Fe and Zn (Hunt).

Objective 2: Determine the effectiveness of current and proposed mineral fortification/supplementation practices for improving mineral nutrition while avoiding excessive or imbalanced mineral intakes. This objective will evaluate the bioavailability of Fe fortificants such as elemental Fe and micronized, encapsulated Fe compounds in human studies (Hunt).

Objective 3: Determine the mechanisms of uptake, transport, and retention of food minerals and how mineral nutritional status influences these mechanisms to impact the bioavailability of essential minerals, non-nutritive metals, and other food components. Cell and whole animal models will be employed to elucidate how the modifications of mineral content of foods can influence the biochemical regulation of specific transporters, cellular trafficking, and interactions of minerals such as Zn, Fe, Cu, Cd, Se, and Mn. (Reeves).

Problem to be addressed with increased funds: Elucidate the roles and diets in support of optimal health and prevention of obesity and related illnesses, cardiovascular disease, osteoporosis and cancer.

Problem to be addressed with increased funds (FY05): Under Performance Measure 4.1.1 of the ARS Strategic Plan and the NP107 Action Plan, this project will develop an enhancement to the food supply by increasing the nutritional value of beef.

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2007

Objective modification FY05: Increase the amount of omega-3 fatty acids in beef to a nutritionally significant level by feeding flax. Demonstrate that the increase in omega-3 fatty acids in the meat are sufficient to have a physiological effect. Study feasibility of increasing selenium in beef to levels that will have an impact on human health when the meat is consumed at recommended levels. This may include studies of organic form of selenium in beef, stability with varying cooking methods, sensory issues, bioavailability and health effects in both steers and consumers.

1b. Approach (from AD-416)

Methodology will include tests of agricultural conditions affecting the amounts and forms of minerals incorporated into in foods; in vitro, cellular, and animal models of mineral transport and absorption; and human experiments with controlled diets to assess mineral absorption, retention, and biological function and to model nutritional requirements.

Specific objectives to be accomplished with increased funding: To study the roles of foods, particularly those produced in the Northern Plains, in the support of health. This work is to be multi-disciplinary, including collaborations such as with the University of North Dakota School of Medicine and Health Sciences and North Dakota State University.

2. Milestones for FY2007

1. Complete feeding portion of human high-Se beef study (1.3)
   Milestone Fully Met

2. Report antioxidants and gene expression study (1.5)
   Milestone Fully Met

3. Report wheat and buckwheat accumulations studies (1.1)
   Milestone Fully Met

4. Complete 2nd year of organic/conventional foods study (1.1)
   Milestone Fully Met

5. Complete high-Se beef and aberrant crypt study (1.3)
   Milestone Not Met
   Reason not met: Critical vacancy (quantitative or qualitative deficiency in personnel
6. Complete Zn requirement study (1.6)
   Milestone Fully Met

7. Report elemental Fe powders study (2.1)
   Milestone Fully Met
   The methodology for the elemental iron powders study was tested and found to be ineffective. However, data from this same study was useful for evaluating the usefulness of serum prohepcidin in predicting human iron absorption, and this was published.

Project Number: 5450-51000-035-00D Accession: 0407991 FY: 2007

8. Complete studies on low mineral intakes, metal transporters, and cadmium accumulation in intestinal cells (3.3)
Milestone Fully Met

9. Complete studies on relationship between Cu deprivation and hephaestin activity (3.4)
Milestone Fully Met

4. Accomplishments

a) Phytic acid inhibition of zinc absorption is unaffected by dietary calcium fortification: Dietary calcium has been regarded as a possible inhibitor of zinc absorption, especially in combination with a diet rich in phytic acid. Women's zinc absorption was measured from diets that contained approximately 700 or 1900 mg/d calcium and 440 or 1800 mg/d phytic acid from foods in all 4 possible combinations. The phytic acid significantly decreased zinc absorption, but the calcium did not, with or without the increased phytic acid. These results suggest that calcium in the range normally consumed does not interfere with zinc absorption, even when products are highly fortified with calcium. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components.)

b) Perennial wheat could be nutritious, less expensive to maintain and more likely to aide the environment than annual wheat: Perennial wheat is emerging as a potential solution to some of these problems caused by annual wheat, such as seed expense, soil cultivation and erosion, and applied fertilizers and chemicals. In collaboration with investigators at the University of Washington, we helped describe a perennial wheat hybrid that yields as much as 93% of the average annual wheat and contains mineral nutrient concentrations of calcium, copper, iron, magnesium, manganese, phosphorus, and zinc that are 46, 45, 31, 27, 38, 33, and 37% higher than annual wheat varieties. Regrowth after harvest is as great as 90% in the perennial variety. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components.)

c) Hemoglobin as the sole source of dietary iron will not support adequate iron status in some animals: Up to ten percent of the iron consumed in the diets of humans comes from heme and hemoglobin, which are major components in blood. Although all animals have specific intestinal heme transporters, some cannot absorb heme iron efficiently; however, they are still used from time to time as experimental models to study the absorption of iron from these sources. This showed that feeding hemoglobin, as the sole source of iron could not maintain normal levels of body iron and caused anemia, and confirmed that the rat is not a good experimental model for the human to study the utilization of iron from hemoglobin or heme. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components.)

5. Significant Activities that Support Special Target Populations

None.

6. Technology transfer

0 Number of new CRADAs and MTAs
0 Number of active CRADAs and MTAs
0 Number of invention disclosures submitted
0 Number of patent applications filed

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2007

0 Number of U.S. patents granted
0 Number of new commercial licenses granted
0 Number of web sites managed
7 Number of non-peer reviewed presentations and proceedings
3 Number of newspaper articles and other presentations for non-science audiences

Scientific Publications: **Log 115:**

1. Sempertegui, F., Estrella, B., Elmieh, N., Jordan, M., Ahmed, T., Rodriguez, A., Tucker, K.L., Hamer, D.H., Reeves, P.G., Meydani, S.N. 2006. Nutritional, immunological, and health status of the elderly population living in poor neighborhoods of quito, ecuador: a preliminary report. British Journal of Nutrition. 96:845-853. **0000182831**

2. Sempertegui, F., Diaz, M., Mejia, R., Rodriguez-Mora, O.G., Renteria, E., Guarderas, C., Estrella, B., Recalde, R., Hamer, D.H., Reeves, P.G. 2007. Low concentrations of zinc in gastric mucosa are associated with increased severity of Helicobacter pylori-induced inflammation. Helicobacter Journal. 12:43-48. **0000199511**

3. Reeves, P.G., Gregoire, B.R., Garvin, D.F., Hareland, G.A., Lindlauf, J.E., Johnson, L.K., Finley, J.W. 2007. Determination of selenium bioavailability from wheat mill fractions in rats by using the slope-ratio assay and a modified Torula yeast-based diet. Journal of Agricultural and Food Chemistry. 55:516-522. **0000199525**

4. Reeves, P.G., Demars, L.C. 2007. Bovine hemoglobin as the sole source of dietary iron does not support adequate iron status in copper-adequate or copper-deficient rats. Nutrition Research. 27:289-294. **0000204353**

5. Swain, J.H., Johnson, L., Penland, J.G., Hunt, J.R. 2007. Electrolytic iron or ferrous sulfate increase body iron in women with moderate to low iron stores. Journal of Nutrition. 137:620-627. **0000173892**

6. Miller, K.B., Caton, J.S., Finley, J.W. 2006. Manganese depresses rat heart muscle respiration. Biofactors. (28)33-46. **0000211694**

7. Lynch, S.R., Bothwell, T., Campbell, L., Cowan, K., Glahn, R.P., Hallberg, L., Hoppe, M., Hulthen, L., Hunt, J.R., Hurrell, R.F., Miller, D., Swain, J., Turner, L., Winichagoon, P., Yeung, C.K., Zeder, C., Zimmermann, M.B. 2007. A comparison of physical properties, screening procedures and a human efficacy trial for predicting the bioavailability of commercial elemental iron powders used for food fortification. International Journal for Vitamin and Nutrition Research. 77:107-124. **0000192625**

8. Beisiegel, J.M., Hunt, J.R., Glahn, R.P., Welch, R.M., Menkir, A., Maziya-Dixon, B.B. 2007. Iron bioavailability from maize and beans: a comparison of human measurements with Caco-2 cell and algorithm predictions. American Journal of Clinical Nutrition. 86:388-396. **0000199746**

Approved: MCGUIRE MICHAEL R **Date:** 09/28/2007

Project Number: 5450-51000-035-18S Accession: 0407722 FY: 2007
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH Principal Investigator: JANET ROSS HUNT
Start Date: 09/29/2003 Term Date: 09/28/2008
National Programs: 107 N Human Nutrition
Title: HUMAN STUDIES RESEARCH
Period Covered From: 10/ 2006 To: 9/ 2007 Final Report? No
Terminate in Two Months? No
Agreement Number: 58-5450-3-0324
Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

1a. Objectives (from AD-416)

To investigate the role of nutrients in human health, to determine their bioavailability from foods and mixed diets, to investigate their biological activities in cancer prevention, in bone and joint health, in cardiovascular health, and in physiological and psychological development and function.

1b. Approach (from AD-416)

1. Provide expert guidance and technical support for human studies to elucidate functions of and quantitative needs for nutrients in maintaining health of adults through reduction of risk factors for cardiovascular disease, diabetes, cancer, osteoporosis and other degenerative diseases.
2. Plan and conduct human studies (including residential, non-residential and field-based investigations) using such approaches as dietary recall, metabolic balance, radio/stable isotope retention, physiological/neurological function assessment, and specific metabolic/enzyme analyses.
3. Design diets to contain known amounts of essential and non-essential nutrients, or foods containing specific nutrients or other bioactive components.
4. Recruit, interview and screen volunteers for eligibility in human studies.
5. Determine clinical chemical (blood constituents), physiological (blood pressure, cardiovascular function, respiratory function, neuro-muscular function), neurological (mood, neurologic function), urinary and fecal excretion, and other measures of biological activity and health status.
6. Publish scientific results.

3. Progress Report

This report serves to document research conducted under a specific cooperative agreement between ARS and the University of North Dakota (UND). Additional details of research can be found in the report for the parent project 5450-51000-035-00D.

Found that individuals with a minor genotype (11% of the population surveyed) for a selenoenzyme, the extracellular glutathione peroxidase (GPX), have plasma selenium levels that are ca. 10% lower than those of the general population. This genotype (Leu/Leu) has been associated with increased risk to lung cancer. This finding raised questions about the use of plasma selenium, the most widely used biomarker of nutritional selenium status, as an indicator of selenium intake and suggests that GPX genotype may be a determinant of response to selenium intake. (Human Nutrition Program (HNP) 107, Component 6: Prevention of obesity and disease: relationship of

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2007

diet, genetics and lifestyle. See the in-house project report 5450-51000-036-00D).
Determined that serum pro-hepcidin, a precursor of the hepcidin molecule hypothesized to regulate iron absorption, was not associated with iron absorption in premenopausal women. (HNP 107, Component 2: Bioavailability of Nutrients and Food Components. See the in-house parent report 5450-51000-035-00D).
Determined that the reduced bioavailability (associated with reduced solubility) of some elemental iron powders can also reduce the enhancing effects of ascorbic acid on their absorption. (HNP 107, Component 2: Bioavailability of Nutrients and Food Components. See the in-house parent report 5450-51000-035-00D).
Found that 20 mg zinc supplementation, 5 days/week for 10 weeks, improved visual memory reaction times, word recognition, and vigilance in cognitive testing of 7th grade students. (HNP 107, Component 4: Nutrient Requirements. See the in-house parent report 5450-51530-009-00D).
Discovered that a high, compared with a low meat diet improved calcium retention in postmenopausal women when calcium intake was low, but had minimal influence when calcium intake was high. (HNP 107, Component 2: Bioavailability of Nutrients and Food Components. See the in-house parent report 5450-51000-034-00D).
Determined that soy protein (substituted for meat protein) had a negligible effect on calcium retention by postmenopausal women. (HNP 107, Component 2: Bioavailability of Nutrients and Food Components. See the in-house parent report 5450-51000-034-00D).
Determined that iron absorption of fortified foods did not differ significantly between genetic carriers (~10% of US population) of a mutation associated with hemochromatosis and those without the mutation. (HNP 107, Component 2: Bioavailability of Nutrients and Food Components. See the in-house parent report 5450-51000-035-00D).
Conducted a survey assessing Native American health, nutrition and physical activity and distributed summaries of the findings to participating Indian tribes and communities. (HNP 107, Component 7: Health Promoting Intervention Strategies for Targeted Populations. See the in-house parent report 5450-51530-009-00D).
Investigators met regularly to discuss/review experimental plans and research outcomes.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 08/20/2007

Project Number: 5450-51000-035-19S Accession: 0408261 FY: 2007
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH Principal Investigator: GERALD F COMBS

Start Date: 04/16/2004 Term Date: 03/15/2008

National Programs: 107 N Human Nutrition

Title: DEVELOPMENT OF RAPID SELENIUM ANALYSIS TECHNOLOGY

Period Covered From: 10/ 2006 To: 9/ 2007 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-4-0346

Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

1a. Objectives (from AD-416)

To develop a prototype device to rapidly measure the selenium concentrations in ground cereals.

1b. Approach (from AD-416)

Design and build a functional prototype to detect selenium in ground samples of air-dry grain suitable for ultimate use in sorting grains for the preservation of high native selenium content. In order that the technology can be ultimately useful in elevators and mills, it will need to meet the following criteria: have a detection accuracy of ca. 0.5 mcg selenium per g of native material; have a turnaround time no greater than 1 hr; and have automatic processing of native, solid phase grains. Acid digestion and vapor extraction methods of sample preparation will be evaluated for combining with two light-absorption methods of selenium detection, hydride generation atomic absorption spectrometry and dye-based colorimetric spectrophotometry, in the resulting liquid or gas phases.

3. Progress Report

This report serves to document research conducted under a specific cooperative agreement between ARS and the University of North Dakota. Additional details of research can be found in the report for the parent project 5450-51000-035-00D, Mineral Utilization and Bioavailability in the 21st Century, with Changing Diets and Agricultural Practices.

A working prototype for a rapid selenium analyzer useful for application with small grains is being developed. The prototype accomplished the rapid liquification of a coarsely ground grain sample using high-pressure steam, followed by the automated instrumental analysis for total selenium content within 50-55 min. The ADODR is monitoring activities to evaluate and discuss the research progress including teleconferences and electronic mail correspondence.

Scientific Publications: Log 115:

Approved: MCGUIRE MICHAEL R Date: 10/03/2007

Project Number: 5450-51000-035-22T **Accession:** 0408646 **FY:** 2007
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH **Principal Investigator:** JAY J CAO
Start Date: 05/01/2004 **Term Date:** 04/30/2009

National Programs: 107 N Human Nutrition

Title: HIGH SELENIUM PINTO BEANS AS A VALUE-ADDED PRODUCT

Period Covered **From:** 10/ 2006 **To:** 9/ 2007 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-4-0418

Organization Name: NORTHARVEST BEAN GROWERS ASSOCIATION

Progress and Outcomes:

1a. **Objectives (from AD-416)**

Determine the selenium content of pinto beans. Determine factors that increase the concentration of selenium in pinto beans. Use GIS data base to model mineral accumulation in pinto beans.

1b. **Approach (from AD-416)**

Pinto bean samples will be obtained at the time of harvest directly from producers in central North Dakota. Beans will be analyzed for minerals including selenium. Data on mineral content will be combined with producer information and data from the GIS data base. These data will be modeled by statistical methods to determine factors that result in selenium accumulation in pinto beans.

3. **Progress Report**

This report serves to document research conducted under a Trust Fund Cooperative Agreement between ARS and NortHarvest Bean Growers, Ltd. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D. The purpose of this agreement was to determine factors that affect the accumulation of selenium in pinto beans. Dry edible beans are a major crop raised in North Dakota, and there may be substantial health benefits associated with consumption of beans. Moreover, beans raised in North Dakota may contain much higher concentrations of selenium, a nutrient that may help prevent cancer. With a change in personnel on this project (i.e., new hire), studies were designed and initiated to evaluate the effects of selenium and other antioxidants in beans on the cellular functions of bone-forming (osteoblasts) and bone-remodeling (osteoclasts) cells in a cell culture system developed for that purpose.

ADODR monitoring activities to evaluate and discuss the research progress included teleconferences and electronic mail correspondence.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 08/20/2007

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2007
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: DAVID M KLURFELD **Principal Investigator:** ERIC O UTHUS
Start Date: 07/21/2004 **Term Date:** 04/30/2009
National Programs: 107 N Human Nutrition
Title: ROLE OF DIETARY SELENIUM ON GENE EXPRESSION, CELL CYCLE AND MOLECULAR MECHANISMS IN CANCER RISK
Period Covered **From:** 10/ 2006 **To:** 9/ 2007 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1a. Objectives (from AD-416)

Determine the molecular and cellular mechanism(s) of action of selenium (Se) in anti-carcinogenesis. Specific objectives include 1) Determine the role of Se in cell cycle progression and apoptosis in models of colon cancer; 2) Determine the role of selenoproteins in cancer prevention and the role of dietary components in the regulation of selenoprotein activity; 3) Determine the mechanism(s) by which Se alters DNA methylation and 4) Determine the relationship of oral selenium intake with selenium status and indicators of cancer risk.

1b. Approach (from AD-416)

A variety of cell culture and animal model approaches will be used. In general, cell culture experiments will be run using cell lines specific for colon. Various forms and concentrations of selenium will be added to serum-free media. Cell growth, indices of selenium status, and indices of cell cycle progression and apoptosis will be measured. These studies will be used to determine the effects of nutritional levels of selenium in supporting cellular survival signaling in human cultured colon cells, and the role of the putative anti-tumorigenic selenium-metabolite, methylselenol, in cell cycle progression and apoptosis in human cultured colon cells. Other cell culture models (colon and/or liver cell lines) will be used in siRNA knockdown studies. These experiments will determine the effect of selenium in cells in which specific genes have been knocked down by siRNA. Other studies will use knock downs of various selenoproteins to determine their role in anticarcinogenicity of selenium. Animal studies will use rats and mice to determine the effects of form and concentration of dietary selenium on 1) selenoprotein expression and activity as related to carcinogenesis, 2) carcinogen-induced aberrant crypt formation (preneoplastic colon cells) and, 3) indices of oxidative stress and one-carbon metabolism including DNA methylation of genomic and gene specific DNA.

2. Milestones for FY2007

1. Optimize the colon cell culture conditions and start a study to determine how cellular selenium status regulates the mitogen-activated protein kinase pathway.
   Milestone Fully Met

2. Optimize the colon cell culture conditions and start a study to test the hypothesis that selenium-induced apoptotic signaling is different in normal versus transformed cells.
   Milestone Fully Met

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2007

3. Determine whether knockdown of thioredoxin reductase (TR) will have minimal functional consequence for the cell because related antioxidant response element (ARE)-regulated antioxidant systems will be compensatorily upregulated.
   Milestone Fully Met
4. Initiate preliminary experiments to test the hypothesis that simultaneous knockdown of TR and a second ARE-regulated protein (ferritin) will severely damage the ARE-regulated antioxidant network and result in severe cellular damage.
   Milestone Not Met
   Reason not met: Critical vacancy (quantitative or qualitative deficiency in personnel
5. Complete methylation assays and gene identification from restriction landmark genomic scanning (RLGS) in the selenium-folate and Ames dwarf mice studies.
   Milestone Substantially Met
   The methodology for RLGS has proven difficult to get running properly resulting in a delay in milestones; this experiment is ongoing.
6. Initiate RLGS study in Ames mice fed various forms of selenium.
   Milestone Not Met
   Reason not met: Other (a reason for not meeting the Milestone other than the ones above)
   The difficulty in getting the RLGS method up and running has delayed this experiment; it will be run FY 2008.
7. Complete development of HPLC-ICP-MS method for selenium in plasma.
   Milestone Fully Met
   The non-protein bound selenium (Se)in plasma, while detectable, has proven to be just at the limit of detection such that separation by physical means (e.g., HPLC) makes detection of components non-quantifiable. For this reason, we have decided to determine whether urinary Se-metabolites, specifically Se-sugars, may be useful as alternative biomarkers of Se status.
8. Conduct selenium intervention trial, including the completion of subject enrollment, conduct interim subject visits and attendant analyses, and compilation of baseline data for all subjects.
   Milestone Fully Met

4. Accomplishments

A cell culture model developed at the Grand Forks Human Nutrition Research Center is useful to assess the biological activity of chemical or food forms of selenium. Assessing the ability of selenium to restore the activity of the selenium-containing enzyme glutathione peroxidase (GPX) is the most common method to determine the biological activity of food or chemical forms of selenium. However, this assessment has mainly been done by using selenium-deficient animals; our goal was to establish a cell culture model. Our model was developed by using gradual reductions in cell media selenium to ultimately arrive at a selenium-deficient state. This non-animal model was able to distinguish the ability of various food or chemical forms of selenium to restore GPX and thus it provides a useful model to study the cellular mechanisms related to the biological activity of selenium. NP107 Human Nutrition: Component 6. Prevention of obesity and disease: relationship between diet, genetics, and lifestyle.

The von Hippel-Lindau tumor-suppressor gene is down-regulated in cells incubated in a low-selenium media. We have hypothesized that one of the mechanisms for the anti-cancer action of selenium is that selenium affects the expression of genes by

affecting DNA methylation. It is known that methylation of DNA will turn off various genes, including tumor suppressor genes. We found that the von Hippel-Lindau tumor-suppressor gene is methylated in cells that are grown with inadequate amounts of selenium in the media. This methylation results in under expression of this gene that is important in suppressing a specific form of cancer. Our results suggest that selenium is needed for proper expression of genes, including certain tumor-suppressor genes. NP107 Human Nutrition: Component 6. Prevention of obesity and disease: relationship between diet, genetics, and lifestyle.

Glutathione peroxidase (GPX) I genotype is a determinant of plasma Se level. We found that the Pro/Pro allelic variant of the extracellular GPX, which has been associated with increased risk to lung cancer, has a prevalence of ca. 11 % in our clinical intervention cohort in Grand Forks, ND, in which it is associated with a 10% lower plasma Se level than that of the general population. This finding is without precedent and challenges the assumption that plasma Se necessarily predicts dietary Se intake and, thus, has direct relevance to the design of dietary surveys including NHANES. NP107 Human Nutrition: Component 6. Prevention of obesity and disease: relationship between diet, genetics, and lifestyle.

The food form of selenium, selenomethionine, is incorporated into proteins and, therefore, produces greater tissue Se levels than inorganic forms of the element. The capacity for tissue Se accumulation from SeMet is determined by the total methionine (Met) content and turnover rates of tissue proteins. That this capacity is exceeded by supranutritional SeMet intakes was indicated by the finding that SeMet-fed animals had only ca. 10% of total muscle Met residues replaced with SeMet residues. NP107 Human Nutrition: Component 6. Prevention of obesity and disease: relationship between diet, genetics, and lifestyle.

5. Significant Activities that Support Special Target Populations

None.

6. Technology transfer

0 Number of new CRADAs and MTAs
1 Number of active CRADAs and MTAs
0 Number of invention disclosures submitted
0 Number of patent applications filed
0 Number of U.S. patents granted
0 Number of new commercial licenses granted
0 Number of web sites managed
7 Number of non-peer reviewed presentations and proceedings
2 Number of newspaper articles and other presentations for non-science audiences

Scientific Publications: Log 115:

1. Azrak, R.G., Cao, S., Pendyala, L., Durrani, F.A., Fakih, M., Combs, G.F., Prey, J., Smith, P.F., Rustum, Y.M. 2007. Efficacy of increasing the therapeutic index of irinotecan, plasma and tissue selenium concentrations is methylselenocysteine dose dependent. Biochemical Pharmacology. 73(9):1280-1287. 0000205673

2. Stranges, S., Marshall, J.R., Natarajan, R., Donahue, R.P., Trevisan, M., Combs, G.F., Cappuccio, F.P., Ceriello, A., Reid, M.E. 2007. Effects of long-term selenium supplementation on the incidence of Type 2 diabetes. Annals of Internal Medicine. 147:217-223. 0000205794

3. Uthus, E.O., Moskovitz, J. 2007. Specific activity of methionine sulfoxide reductase in CD-1 mice is significantly affected by dietary selenium but not 0000190405

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2007

zinc. Biological Trace Element Research. 115:265-275.

4. Uthus, E.O., Ross, S.A. 2007. Dietary selenium affects homocysteine metabolism differently in Fisher-344 rats and CD-1 mice. Journal of Nutrition. 137:1132-1136. **0000204963**

Approved: MCGUIRE MICHAEL R **Date:** 09/28/2007

Project Number: 5450-51000-036-02R **Accession:** 0410110 **FY:** 2007
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: DAVID M KLURFELD **Principal Investigator:** GERALD F COMBS
Start Date: 09/01/2005 **Term Date:** 08/31/2010

National Programs: 107 N Human Nutrition

Title: SELENIUM NUTRITION IN HUMANS: PREDICTING DIETARY SELENIUM NEEDS TO ACHIEVE TARGET BLOOD SELENIUM LEVELS

Period Covered **From:** 10/ 2006 **To:** 9/ 2007 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 05-5450-5-0330

Organization Name: NATIONAL CANCER INSTITUTE, DEPARTMENT OF HEALTH AND HUMAN SERVICES, NATIONAL INSTITUTES OF HEALTH

Progress and Outcomes:

1a. Objectives (from AD-416)

Develop algorithm relating increase in stable plasma Se level to that at baseline and level of supplemental Se.

1b. Approach (from AD-416)

Conduct a randomized, double-blind, intervention study will be conducted with healthy men (120) and women (120) randomized to 0, 50, 100, or 200 ug Se/day (as L-selenomethionine) administered in daily oral doses. Fasting blood samples and urine samples will be drawn two wks prior to and periodically throughout the 1-yr study. The following measurements will be made: Se, homocysteine, vitamin B12 and folate in plasma; Se and 8a-deoxyquanosine in urine; DNA damage and allelic variants of Se-dependent enzymes in lymphocytes. Results will be used to compute the relationship of final-plateau plasma (9-12 mos.) Se concentration as a function of baseline (0 mos.) Se level, Se dose, metabolic body size and urinary Se, as well as outcomes related to carcinogenesis.

3. Progress Report

This report serves to document research conducted under an interagency reimbursable agreement between ARS and National Cancer Institute of the National Institutes of Health. Additional details of research can be found in the report for the parent project 5450-51000-036-00D.

This collaboration planned and implemented the first clinical intervention trial using nutritional doses of selenium, the "LoDoSe trial", which was initiated in FY2006 and will continue through FY2008. This trial will provide the fundamental information to allow the projection of daily dietary selenium needs from plasma selenium levels found to be thresholds for such health effects as minimalization of cancer risks in healthy Americans, which prospect has direct implications to the US food industry which can produce foods capable of providing 50-100 mcg selenium per day through geographic sourcing, or selenium-fortification. This year, 253 adult men and women volunteers participated in the intervention, with all completing the 1-yr intervention phase of the trial by the end of FY 2007. Studies of the baseline characteristics of the cohort showed that: i) none were deficient in selenium (with plasma selenium levels less than 80 ppb); ii) the cohort average plasma selenium level, 141 ppb, was slightly greater than that of the NHANES-III cohort; iii) plasma

Project Number: 5450-51000-036-02R **Accession:** 0410110 **FY:** 2007

selenium level was positively associated with the consumption of dairy products, fish, and fish other than tuna, as estimated from food frequency questionnaire methodology; and iv) individuals with the Leu/Leu genotype for extracellular glutathione peroxidase constituted 11% of the cohort and had plasma selenium levels ca. 10% lower that the cohort average. ADODR monitoring activities to evaluate and discuss the research progress included teleconferences and electronic mail correspondence.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 08/20/2007

FINAL PROGRESS REPORTS

OF

TERMINATED CRIS WORK UNITS

Project Number: 5450-51530-009-01T **Accession:** 0406520 **FY:** 2007
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH **Principal Investigator:** HENRY C LUKASKI
Start Date: 10/01/2002 **Term Date:** 09/30/2007

National Programs: 107 N Human Nutrition

Title: DETERMINATION OF THE EFFECTS OF BUCKWHEAT ON MANAGEMENT OF PRE-DIABETES AND NON-INSULIN DEPENDENT DIABETES (NIDDM) IN ANIMAL MODELS

Period Covered **From:** 10/ 2006 **To:** 9/ 2007 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-3-0402

Organization Name: MINN-DAK GROWERS, LTD.

Progress and Outcomes:

1a. **Objectives (from AD-416)**

Determine the usefulness (e.g., dose and efficacy) of buckwheat in reducing the risk of development and improving the management of NIDDM and Metabolic Syndrome.

1b. **Approach (from AD-416)**

Studies will evaluate the effect of graded doses of fagopyritols from buckwheat to ameliorate the development of NIDDM by using various rodent models. Weanling, male Zucker rats will be fed low and high buckwheat diets; fasting blood glucose and insulin will be monitored serially, and glucose tolerance test will be administered to test the hypothesis that buckwheat-derived fagopyritols reduce the risk of development of NIDDM in rodents susceptible to NIDDM. Another study will determine the effects of graded dietary buckwheat on maintenance of glucose and insulin homeostasis of rodents with chemically-induced diabetes. These studies will provide key evidence necessary to plan human trials of the efficacy of buckwheat in reducing risk of NIDDM.

3. **Progress Report**

This report serves to document research conducted under a Trust Fund cooperative Agreement between ARS and Minn-Dak Growers, Ltd. Additional details of research can be found in the report for the parent project, 5450-51530-009-00D, Micronutrient Roles in Physiology and Health.

We had planned a trial to evaluate the effect of a commercial extract of buckwheat, including fagopyritols, on insulin sensitivity of male, Zucker obese (ob/ob). Our collaborator was unable to provide the extract with adequate concentrations of a mixture of fagopyritols to undertake the study. Until this extract is available, the study will not proceed. Collaborators met frequently to discuss project plans and approaches to resolve the problem of a lack of appropriate standards.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 09/28/2007

Project Number: 5450-51530-009-02N **Accession:** 0408500 **FY:** 2007
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH **Principal Investigator:** JAMES G PENLAND
Start Date: 10/18/2004 **Term Date:** 04/16/2007

National Programs: 107 N Human Nutrition

Title: NUTRITION EFFECTS ON COGNITIVE PERFORMANCE OF YOUNG VERSUS ELDERLY COMMUNITY-LIVING ADULTS

Period Covered From: 10/ 2006 **To:** 9/ 2007 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-5-0101N

Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

1a. **Objectives (from AD-416)**

The objective of this cooperative research project is to determine whether micronutrient intakes and status are factors mediating age differences in memory, especially age-related declines in verbal memory.

1b. **Approach (from AD-416)**

Previous research indicates that reasoning, vocabulary, gender and vitamin nutrition (e.g., B12) moderate age-related changes in memory performance. The proposed research will determine whether dietary intakes and status of mineral nutrients (e.g., zinc and magnesium) are additional mediating factors in the memory performance of older and younger adults. Thirty older (60-75 y) and thirty younger (21-35 y) healthy, community-dwelling adults (males and females) will participate in this study. Estimates of mineral intakes and status will be obtained from an analysis of participant responses to a food frequency questionaire and 24-hour dietary recall interview, and a blood sample. Memory preformance will be measured using a prose memory task, a word recall task and two measures of working memory. Reasoning and vocabulary skills will also be determined by standardized tasks.

3. **Progress Report**

This report serves to document research conducted under a non-funded cooperative agreement between ARS and the University of North Dakota. Additional details of research can be found in the report for the parent project 5450-51530-009-00D, Micronutrient Roles in Physiology and Health.

Memory performance may be moderated by many factors including age, gender, level of depression, and nutritional status, particularly vitamin B12. Other nutrients, such as zinc and magnesium, have been described as other potentially modifying factors. Young (21-35 year old) and elderly (60-85 years old) adults were examined to determine nutritional factors that moderated memory performance in adults living in the community. Results confirmed age-related changes in word list recall, story recall, and working memory span. Although zinc and magnesium intakes were associated with memory performance, only vitamin B12 status was significantly related to this aspect of brain function.

ADODR monitoring: Investigators met regularly at the Center and University of North Dakota to review experimental plans and data, and prepare final report

Project Number: 5450-51530-009-02N Accession: 0408500 FY: 2007

(dissertation).

Scientific Publications: Log 115:

Approved: MCGUIRE MICHAEL R Date: 09/28/2007

Project Number: 5450-51000-036-01R Accession: 0406009 FY: 2007
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: DAVID M KLURFELD Principal Investigator: ERIC O UTHUS
Start Date: 07/15/2002 Term Date: 06/30/2007
National Programs: 107 N Human Nutrition
Title: DETERMINATION OF NUTRIENT EFFECTS ON CANCER SUSCEPTIBILITY ON EPIGENETIC PROCESSES IN ANIMAL MODELS
Period Covered From: 10/ 2006 To: 9/ 2007 Final Report? Yes
Terminate in Two Months? No
Agreement Number: 02-5450-2-0217
Organization Name: NATIONAL CANCER INSTITUTE, DEPARTMENT OF HEALTH AND HUMAN SERVICES, NATIONAL INSTITUTES OF HEALTH

Progress and Outcomes:

1a. Objectives (from AD-416)

The purpose of this research is to examine the interactive effects of dietary selenium and folate on cancer susceptibility, global and gene specific DNA methylation, DNA methyltransferase activity, and one-carbon metabolism using an animal system. This research will consist of exploratory work to determine the feasibility of various animal models (Big Blue rats/mice, methionine sulfoxide reductase knockout mice, DNA methyltransferase knockout mice) on cancer susceptibility as measured by mutation frequency in vivo, global and gene specific DNA methylation, DNA methyltransferase activity, and one-carbon metabolism (homocysteine, methionine, S-adenosylmethionine, S-adenosylhomocysteine, cysteine, and glutathione concentrations as well as the enzymes involved in their metabolism) in various tissues.

1b. Approach (from AD-416)

Animals (rats or mice) will be fed diets containing various amounts and/or forms of selenium in combination with other nutrients of interest (e.g., folate, zinc). Tissues will be collected and various parameters (indices of cancer susceptibility including DNA methylation or factors that can affect DNA methylation) will be measured and correlated to dietary intake of selenium (and folate or zinc, for example). Initial experiments will most likely use Big Blue rats or mice. These animals are used for studying in vivo mutagenesis and may be found to be extremely useful models in nutrition-based cancer research.

3. Progress Report

This report documents research conducted under a reimbursable agreement between ARS and the National Cancer Institute. Additional details of this research can be found in the report for the in-house associated project 5450-51000-031-00D, Role of Selenium on Gene Expression, Cell Cycle and Molecular Mechanisms in Cancer Risk.

The purpose of this agreement is to examine the interactive effects of dietary selenium and folate in cancer susceptibility, global and gene specific DNA methylation, DNA methyltransferase activity, and one-carbon metabolism using animal models. This work supports that done in 5450-51000-031-00D, specifically objectives 2 (Determine the role of selenoproteins in cancer prevention and the role of dietary components in the regulation of selenoprotein activity) and 3 (Determine the

Project Number: 5450-51000-036-01R **Accession:** 0406009 **FY:** 2007

mechanism(s) by which Se alters DNA methylation).

One of the main hypotheses of this project is that selenium affects promoter region DNA methylation and hence gene regulation. Previously, we have used the candidate gene approach or a methylation array (with a limited number of genes queried) to test this hypothesis. We found that the von Hippel-Lindau (VHL) tumor-suppressor gene was methylated in the promoter region in cells grown in a selenium-deficient media. This methylation resulted in a down-regulation of this gene as determined by real time PCR. In conjunction with candidate and array-based approaches we are using restriction landmark genomic scanning (RLGS) to determine whether selenium affects DNA promoter region methylation. This approach allows the screening of close to 2000 genes at a time. Currently we are using RLGS on genomic DNA from liver, kidney and brain of rats fed 0, 0.2 or 2.0 ug Se/g diet with selenium as either selenite or Se-methyl-selenocysteine. We are also running RLGS on DNA from Caco-2 cells grown in a serum-free media containing 0 (deficient) or 250 (control) nM Se (as Se-methyl-selenocysteine). Additionally, RLGS is being used on samples from Ames dwarf mice. In addition to their long life, these mice also have a low incidence of cancer. Previously, we determined that their methyl/methionine metabolism is markedly different compared to wild-type controls suggesting that this might be a possible explanation for their lower cancer incidence. We are using RLGS on tissues from the Ames dwarf to determine if there are genes that are differentially methylated; if there are differences (compared to wild-type), we will determine if these genes are important in cancer risk reduction,

The project involving the Big Blue rodent model is nearing completion. This model is being used to evaluate spontaneous mutations as affected by dietary selenium. We will determine whether there are differences in spontaneous mutations in DNA from liver and colon among groups of rats fed one of three diets: deficient, adequate, or supranutritional in selenium.

ADODR monitoring activities: Involved parties at the GFHNRC and NCI stay in contact via phone, email and in person (at scientific meetings) to discuss the progress of project.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 10/01/2007